Citation:

Budek AZ, Hoppe C, Michaelsen KF, Mølgaard C. High intake of milk, but not meat, decreases bone turnover in prepubertal boys after seven days. *Eur J Clin Nutr.* 2007 Aug; 61 (8): 957-962.

PubMed ID: 17228345

Study Design:

Non-Randomized Controlled Trial

Class:

C - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To compare the short-term effect of a high milk and a high meat intake, identical in the amount of protein, on markers of bone formation and bone resorption in pre-pubertal boys.

Inclusion Criteria:

- Male gender
- Eight years old
- Healthy with normal growth
- Habitual daily milk intake >500ml
- Does not take medications known to affect growth and bone metabolism
- Written consent of subject's parents.

Exclusion Criteria:

None.

Description of Study Protocol:

Recruitment

Eight-year-old boys were randomly recruited through the Central Personal Register from Copenhagen and Frederiksberg area between September and October 2000.

Design

Non-randomized trial; researchers assigned the first (14) eligible subjects to the milk intervention and the second (14) eligible subjects to the meat intervention group.

Dietary Intake/Dietary Assessment Methodology

- Three-day weighted food records (two weekdays and one weekend day) were keep for the three days preceding the intervention, and for the last three days of the study
- Average daily intake of energy and selected nutrients was calculated for each subject using Danish food composition database (DANKOST 2000, Dansk Catering Center, Herlev, Denmark).

Blinding Used

Not used.

Intervention

- Subjects were instructed to consume either 1.5 liter of skimmed milk per day or 250g of low-fat meat per day for seven days
- Each treatment was designed to add approximately 53g of protein to the diet. Otherwise, subjects were instructed to maintain their normal dietary intake during the study.

Statistical Analysis

- Unpaired two-tailed Student's T-test (significance $P<0.05$) was used to compare baseline variables (anthropometric measures, age, select dietary nutrients and serum markers of bone turnover) and changes in weight and selected dietary variables over the intervention between the two treatment groups
- Separate multiple linear regression models were constructed to test the effect of treatment on end-of intervention concentrations of bone markers. These models were adjusted for baseline value of each dependent variable
- Kruskal-Wallis was used to test the effect of treatment on change in bone markers (from baseline to end-of intervention) to confirm the results of the linear regression models.

Data Collection Summary:

Timing of Measurements

Measures were taken from samples obtained between 8 a.m. and 10 a.m. on day zero (baseline) and day seven (end of intervention).

Dependent Variables

- Serum bone-specific alkaline phosphate (s-BAP): Measured in serum by ELISA in duplicate
- Serum C-terminal telopeptides of type I collagen (s-CTX): Measured in serum by ELISA in duplicate
- Serum osteocalcin (s-OC): Measured in serum by automated chemiluminescent immunoassay
- Weight
- Select nutrients: Estimated by food record. Nutrients included: Total energy, protein, fat, carbohydrate, calcium, phosphorus, vitamin D and magnesium.

Independent Variables

Treatment (high milk or high low-fat meat diet).

Control Variables

- Body mass index (BMI)
- Based on differences in baseline values, analysis were adjusted for baseline carbohydrate and fat intake, and insulin-like growth factor binding protein 3
- Analysis examining follow-up bone marker measures adjusted for baseline values of given marker.

Description of Actual Data Sample:

- *Initial N:* 28 (14 per group, all males)
- *Attrition (final N):* 24 (12 per group)
- *Age:* Eight years old
- *Ethnicity:* Not reported
- *Other relevant demographics:* None
- *Anthropometrics:*
  - Baseline carbohydrate and fat intake (as a percentage of total energy intake)
  - Baseline insulin-like growth factor were significantly different between treatment groups
- *Location:* Out patient study performed at the University of Copenhagen, Denmark.

Summary of Results:

- At baseline, the groups did not differ with respect to serum concentrations of bone markers
- After seven days, s-OC and s-CTX were significantly reduced in the milk group compared to the meat group
- s-BAP decreased in both groups, but there were no significant differences between the groups.

Baseline and Day Seven Concentrations of Bone Markers by Treatment Group

Bone Turnover Marker	Milk Group (N=12)		Meat (N=12)		P-value for Difference in Day Seven Markers Between Treatment Groups
	Baseline	Day Seven	Baseline	Day Seven	
s-OC (ng/mL)	51.1±11.9	35.3±9.4	51.2±14.8	54.5±17.1	0.003
s-BAP (U/L)	141.0±19.3	135.5±19.0	139.7±19.2	129.2±22.1	0.06
s-CTX (ng/mL)	2.19±0.5	1.78±0.4	1.99±0.4	1.97±0.4	0.04

Baseline and Day Seven Intake of Select Nutrients by Treatment Group

Nutrient	Milk Group (N=12)		Meat (N=12)		P-value for Difference in Baseline Intake Between Treatment Groups	P-value for Change in Dietary Intake (Day Seven Baseline) Between Treatment Groups
	Baseline	Day Seven	Baseline	Day Seven		
Carbohydrate (percent of energy intake)	51.4±3.5	51.8±4.1	56.6±4.8	46.8±8.2	P<0.005	0.0003
Fat (percent of energy intake)	34.6±4.1	26.7±4.1	30.0±4.8	32.5±5.5	P<0.05	<0.0001
Calcium (grams per day)	1.0±0.3	2.9±0.2	0.9±0.2	0.8±0.2		<0.0001
Phosphorus (grams per day)	1.4±0.3	2.8±0.3	1.3±0.2	1.4±0.3		<0.0001
Ca:P ratio (mg/d:mg/d)	0.7±0.1	1.0±0.1	0.7±0.1	0.6±0.1		<0.0001
Magnesium	263±70	442±81	256±47	257±61		<0.0001

Other Findings

Boys in the milk group increased body weight by 0.54kg compared with the boys in the meat group, that did not exhibit a change body weight (P=0.003).

Author Conclusion:

At the equal protein intake, milk, but not meat, decreased bone turnover in pre-pubertal boys after seven days.

Reviewer Comments:

- *The study covered a very limited time period for dietary intervention; it is hard to determine what the long-term implications of such an intervention (or similar dietary patterns of food consumption) would be on bone turnover or bone mass density*
- *This intervention follows a very limited number of subjects and limited demographic information is reported for subject participants*
- *This intervention only used male subjects, and it is unclear whether differences would have*

been seen had females been included.

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	???
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes

3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	No
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	No
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	No
4.4.	Were reasons for withdrawals similar across groups?	???
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	No
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes

6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	???
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	???
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes

8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes